The term "specifically binding to" or "specifically recognizing" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with an association constant (Ka) of at least about 1 x ~~10^6~~ 10^6 M-1 or ~~10^7~~ 10^7 M-1, or about ~~10^8~~ 10^8 M-1 to ~~10^9~~ 10^9 M-1, or about ~~10^10~~ 10^10 M-1 to ~~10^11~~ 10^11 M-1 or higher, and binds to the predetermined antigen with an affinity that is at least two-fold greater than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "an antibody recognizing an antigen" and " an antibody specific for an antigen" and "an antibody which binds specifically to an antigen" are used interchangeably.

Nucleic Acids

This invention provides pharmaceutical compositions comprising MAFA binding and MAFA ligand-binding polypeptides, e.g., soluble MAFA polypeptides, and anti-MAFA antibodies, and epitope binding fragments thereof, and the recombinant or isolated nucleic acids that encode them. Accordingly, the invention provides means to make and express those nucleic acids. As the genes and vectors of the invention can be made and expressed in vitro or in vivo, the invention provides for a variety of means of making and expressing these genes and vectors. One of skill will recognize that desired phenotypes associated with altered gene activity can be obtained by modulating the expression or activity of the genes and nucleic acids (e.g., promoters) within the vectors of the invention. The invention can be practiced in conjunction with any method or protocol known in the art, which are well described in the scientific and patent literature.

General Techniques

The nucleic acid sequences of the invention and other nucleic acids used to practice this invention, whether RNA, cDNA, genomic DNA, vectors, viruses or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed recombinantly. Any recombinant expression system can be used, including bacterial cells, mammalian, yeast, insect or plant cell expression systems.

Alternatively, these nucleic acids can be synthesized in vitro by well-known chemical synthesis techniques, as described in, e.g., Carruthers (1982) Cold Spring Harbor Symp. Quant. Biol. 47:411-418; Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997)

by manipulating MAFA-associated cellular activities, can be used to regulate functions of NK cells and T cells.

Materials and methods

Animals: Fisher and S.D. rats were purchased from Harlan (Indianapolis, IN). C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, MA).

Antibodies and cells: Anti-HA antibody, anti-His antibody, and anti-His agarose were purchased from Babco. Anti-FLAG (M2) antibody and anti-FLAG agarose were purchased from SIGMA (St Louis, MO). Anti-DX5 antibody-PE conjugated and anti-CD16/CD32 antibody were purchased from Pharmingen. Anti-rat IgG antibody-PE conjugated was purchased from Southern Biotechnology Associates, Inc. HEK293 cells and RBL cells were a gift from Dr. Carl Ware and Dr. Yun-Cai Liu, respectively. 3Y1 cells were obtained from RIKEN (Tokyo). VSV peptide-specific mouse CTL lines were generated as previously described by Franco (1999) J. Immunol. 162:3388-3394.

cDNA cloning of mouse MAFA: Mouse spleen QUICK-Clone cDNA (CLONTECH) was used as a template, and PCR was performed to amplify mouse MAFA cDNA fragment with primers (5'-CCTTGTGATGGTGGCTTTGGGGCTTTTGACTG-3' (SEQ ID NO:7) and 5'-ACTGCAAAGCAACCTCACAACTGGAGGC-3') (SEQ ID NO:8) from the rat MAFA DNA sequence (SEQ ID NO:6) at ~~95°C~~ 95°C for 5 sec, ~~55°C~~ 55°C for 30 sec and ~~72°C~~ 72°C for 2 min for 30 cycles. The amplified cDNA fragment was labeled with 32P using the Prime-It II random primer labeling kit (Stratagene, San Diego, CA) and used as a probe for screening a mouse spleen cDNA library (Stratagene) using plaque hybridization, as described by Sambrook. One clone, containing a nearly full-length cDNA encoding murine MAFA, with the exception of four amino acids at the N-terminus, was obtained.

Expression and production of MAFA protein: To express FLAG-tagged MAFA, a cDNA encoding amino acid residues 64 to 188 of the extracellular domain of mouse MAFA was amplified by PCR using the cloned mouse MAFA cDNA as a template with primers (5'-ATATGGATCCTCCAAGGACTCTACATGTTC -3' (SEQ ID NO:9) and 5'-ATATGCGGCCGCTCAGTATAGGACCTTCTTACAG -3' (SEQ ID NO:10) and inserted into pFastBac donor plasmids (Gibco BRL) at the 3'-end of a honey bee melittin signal peptide and

The inhibitory effect of the monoclonal antibody 7B5 was quite dramatic. In spite of the presence of other inhibitory receptors on NK cells, 7B5 antibody alone exhibited a strong inhibitory effect on NK cell function. This demonstrates that signaling through MAFA is sufficient to inhibit the cytotoxic activity of NK cells and this is independent from other NK cell receptors such as Ly49. It should be noted that only a limited number of NK cells cultured with IL-2 were MAFA+. It is possible that only a fully-activated NK cell subset expresses MAFA. The present study also demonstrated that clustering of FcεRI is not required for the induction of MAFA signaling since NK cells do not express FcεRI.

It was also demonstrated that recombinant soluble MAFA augmented NK cell function. This demonstrates that the soluble MAFA interfered with the interaction between MAFA and its ligand, thus inhibiting signaling through the MAFA receptor. The possibility that soluble MAFA prevents the interaction between other C type lectin receptor(s) and their ligands cannot be excluded because MAFA is a lectin receptor and soluble MAFA does bind to glycoproteins derived from fungi. However, it is unlikely that the MAFA ligand is the same ligand as for other NK cell receptors, which have been identified as MHC Class I specific receptors, since MAFA inhibits IgE-stimulated mast cell activation, which is independent of MHC Class I interaction.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.